

NORMALISE

Inhibition of PD-1 to Restore Monocyte/Macrophage Function in Liver Failure

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Name & Role

Date

Signature

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Clinical Queries

Clinical queries should be directed to the local PI and his / her study team who will direct the query to the appropriate person.

Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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Medical Research Council (MRC)

This protocol describes the NORMALISE study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

AD	Acute decompensation of cirrhosis
AE	Adverse Event
ALF	Acute liver failure
ALT	Alanine Aminotransferase
AR	Adverse Reaction
AST	Aspartate Aminotransferase
BP	Blood Pressure
CI	Chief Investigator
CRF	Case Report Form
CXR	Chest X-ray
DMEC	Data Monitoring and Ethical Committee
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
GI	Gastrointestinal
ICMJE	International Committee of Medical Journal Editors
IUD	Intrauterine Device
i.v.	Intravenous
LDH	Lactate Dehydrogenase
MA	Marketing Authorisation
MELD	Model for End Stage Liver Disease
MSU	Mid-stream urine
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PIS	Patient Information Sheet
QA	Quality Assurance
REC	Research Ethics Committee

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
s.c.	Subcutaneous
SIRS	Systemic Inflammatory Response Syndrome
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
UTI	Urinary Tract Infection
WHO	World Health Organisation

KEYWORDS

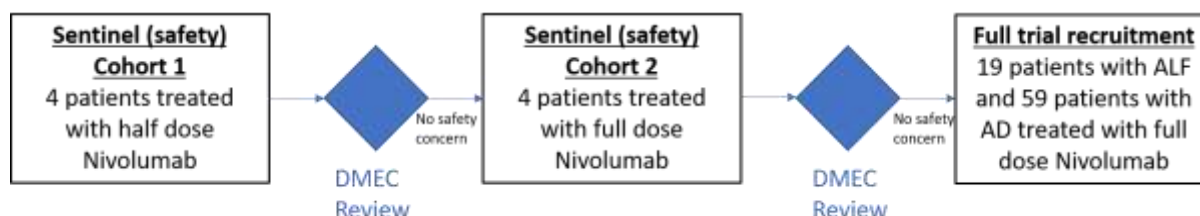
Acute liver failure, acute decompensation of cirrhosis, infection, immunology, bacteraemia

STUDY SUMMARY

TITLE	Inhibition of PD-1 to Restore Monocyte/Macrophage Function in Liver Failure (NORMALISE)
DESIGN	Non-CTIMP, basic science study involving procedures with human participants
AIMS	To determine whether treatment with an anti-PD1 antibody will reverse the observed immunological defect in phagocytosis in PD1 expressing monocytes in patients with liver failure.
OUTCOME MEASURES	The primary endpoint is the change in HLA-DR expression in peripheral blood monocytes at day 15.
POPULATION	Acute liver failure (ALF) and acute decompensated cirrhosis patients (AD)
ELIGIBILITY	<i>Inclusion criteria – ALF</i> • Male and female patients aged 18 years or older at screening • Clinical diagnosis of acute liver failure: ○ Jaundice (Bilirubin > 40 uMol/L) ○ INR > 1.5 ○ Any degree of encephalopathy ○ No history of cirrhosis or advanced chronic liver disease • Informed consent (provided by a relative or a professional legal representative when patient lacks capacity) <i>Inclusion criteria - AD</i> • Male and female patients aged 18 years or older at screening • Clinical diagnosis of acute decompensation of cirrhosis including acute-on-chronic liver failure characterised by at least one of: ○ Ascites ○ Spontaneous bacterial peritonitis ○ Encephalopathy (any degree) ○ Variceal haemorrhage • Evidence of cirrhosis based on any of the following: ○ Liver biopsy (at any time) ○ Elastography (at any time) ○ Radiological imaging (at any time) • Informed consent (provided by the patient or a relative or a professional legal representative)
DURATION	18 months

REFERENCE DIAGRAM

OVERALL STUDY SCHEMA FOR THE NORMALISE STUDY



1. INTRODUCTION

1.1. Background

Acute liver failure (ALF), acute decompensated cirrhosis (AD) and alcohol-related hepatitis are all associated with increased susceptibility to infection which invariably leads to further deterioration in liver function. Acute decompensation may progress to acute-on-chronic liver failure (ACLF) where liver failure is accompanied by failure in other organs (kidney, heart, lung and brain); multi-organ failure is associated with high mortality rates (6). In ALF, bacteraemia occurs in 35% of patients with approximately equal distribution of Gram positive and Gram negative organisms (1). In patients admitted to hospital with cirrhosis 25 - 46% have infection which is associated with a four-fold increased risk of mortality. Excess incidence of bacterial infection coupled with dysregulated inflammatory responses in patients with cirrhosis induce circulatory dysfunction, acute decompensation and ACLF (7). Amongst patients with cirrhosis, the prevalence of multi-drug resistant organisms is 23 - 39% indicating that novel approaches are required to combat the risk of infection (2). A number of defects in innate and adaptive immune responses have been described in patients with ALF, decompensated cirrhosis and alcohol-related hepatitis which provide a partial explanation for some of the observed immune paresis. However, the importance of each individual immunological defect has been difficult to judge until now due to the absence of a targeted therapy.

1.2. Rationale For Current Study

Animal models and in vitro experiments do not recapitulate the complexity of human disease, particularly in liver disease. Whilst our previous work in animal models and ex-vivo monocytes strongly implicate the PD-1 pathway in the immune paresis observed in both acute liver failure and in acute decompensation of cirrhosis, it cannot be assumed that inhibition of PD-1 will be as effective in restoring immune function in vivo. Moving cautiously into human studies using a detailed experimental approach is the conventional next step in the progression from our basic immunology to a potential therapeutic intervention. Although for practical and ethical reasons we

are unable to directly assess Kupffer cell/macrophage responses in humans, we know that in mice and through cross sectional studies in humans, that monocyte phenotype and function reflect macrophage function in the liver. This provides an accessible and practical method of assessing the response to therapy with the anti-PD1 antibody.

Given the concerns about the potential safety of anti-PD1 treatment an experimental medicine approach provides an ideal opportunity to monitor adverse events carefully in a tightly controlled environment.

1.3. Rationale For This Study Population

ALF is a relatively rare disorder so it would be difficult to conduct a large scale study with clinical endpoints in this population without evidence of the desired immunological effect in vivo. AD is not such a rare disorder and our unpublished data show that patients with this condition have the same phagocytic problem as patients with ALF.

The primary aim of this research is to determine whether the observed immunological defect in phagocytosis in PD1 expressing monocytes can be reversed. High levels of HLA-DR -ve/PD-1 +ve monocytes with phagocytic defects have been observed in both ALF and AD patients and the current project design will provide information on the immunological response to anti-PD1 inhibition in both scenarios. There is no plan to directly compare the two groups of patients.

As autoimmune liver disease is one cause for ALF, this group of patients will be excluded from the study as the checkpoint inhibitor may potentially exacerbate the liver injury.

1.4. Rationale For Measuring HLA-DR Expression

The assay for phagocytosis shows a high level of batch-to-batch variation and therefore not an ideal primary endpoint measurement. We and others have demonstrated low levels of HLA-DR on circulating monocytes in patients with liver failure; the lowest levels were found in those patients who developed sepsis (8,9). We now know that PD-1 positive monocytes with impaired phagocytosis have low levels of HLA-DR with a strong inverse correlation between the two markers. Furthermore, we have found in ex-vivo experiments that inhibition of PD-1 leads to increased HLA-DR expression in addition to restoration of phagocytic function. In patients with sepsis, where similar immunological defects are observed, checkpoint inhibition with anti-PD-L1 restores HLA-DR expression (10).

1.5. Rationale For 16S-DNA Measurement and Prophylactic Antibiotics

Circulating bacteria and bacterial products are detectable in patients with AD using a highly sensitive 16S-rDNA real-time PCR assay. We have previously shown that 16S-rDNA levels indicate susceptibility to infection (3). As restoration of monocyte/macrophage function would be expected to suppress 16S-rDNA, this assay will be used as an additional validation for the desired therapeutic effect.

1.6. Rationale For Safety Cohorts

The monoclonal antibody targeting PD-1 (Nivolumab) is licensed for use in melanoma, lung cancer, renal cell carcinoma, Hodgkin lymphoma, head and neck cancer, colon cancer, and liver cancer. The drug works through checkpoint inhibition thereby releasing tumour-specific lymphocytes to target cancer cells.

Therapeutic checkpoint inhibition is associated with immune mediated tissue injury in a minority of patients. Whilst this does include hepatic inflammation, only in anecdotal cases has impairment of liver function been observed. Safety data will be collected and monitored through sentinel (safety) cohorts.

There will be two sentinel cohorts. In the first sentinel cohort four patients with acute decompensation will be given 50% (120 mg) dose of Nivolumab. These patients will be monitored carefully for any unexplained deterioration of liver function and for signs of any of the other common side effects of checkpoint inhibitor therapy. These adverse events would normally occur 6-12 weeks (maximum 24 weeks) after treatment so this sentinel cohort will be monitored for 3 months. In the event of unexplained deterioration of liver function liver biopsy will be used to determine causality (11). Immunological studies will also be performed in these patients. Once the first cohort has been assessed a second sentinel cohort will be treated with a full dose of Nivolumab (240 mg) and assessed over a 12 week period. The study DMEC will be asked to review outcome and safety data once the first sentinel cohort has completed 12 weeks of follow up and when the second sentinel cohort has completed 12 weeks of follow up before moving into the next phase of the study.

2. STUDY OBJECTIVES

To determine whether treatment with an anti-PD1 antibody will reverse the observed immunological defect in phagocytosis in PD1 expressing monocytes in patients with liver failure.

3. STUDY DESIGN

Non-CTIMP, physiological assessment in two groups of patients: 1. Patients (N = 19) with ALF and 2. Patients (N=59) with AD. The study will follow patients up for 30 days. Patients should commence treatment within 48 hours of study enrolment. Anti-PD1 antibody (Nivolumab) will be administered as a single 240 / 200 mg dose (120 / 100 mg dose in the first sentinel cohort patients) diluted in 100 ml of 0.9% saline and administered intravenously over 30 minutes.

3.1. Sentinel Cohorts

Prior to full recruitment to the study, two sentinel cohorts of 4 patients will be run, each for 12 weeks. In the first sentinel cohort, patients with either acute liver failure or acute decompensation will be treated with a half dose of study drug (120 /100 mg) and followed for a total of 12 weeks. These patients will be monitored carefully for any unexplained deterioration of liver function and for signs of any of the other common side effects of checkpoint inhibitor therapy (colitis, thyroid dysfunction, skin rash or

pneumonitis). These adverse events would normally occur 6-12 weeks after treatment (maximum 24 weeks). In the event of unexplained deterioration of liver function liver biopsy will be used to determine causality (11). Immunological studies will also be performed in these patients. The DMEC will monitor the safety data obtained in this first cohort.

Assuming the committee do not find evidence of harm a second sentinel cohort of four patients will be treated with a full dose (240 / 200 mg) study drug and followed for a total of 12 weeks. The DMEC will monitor the safety data and if satisfied with the safety data, the study will proceed to recruitment to the main study. All patients will receive a single dose of the drug Nivolumab.

3.2. Study Outcome Measures

3.2.1. Primary Outcome

The primary endpoint will be the change in HLA-DR expression in peripheral blood monocytes at day 15.

3.2.2. Secondary End Points

- Monocyte phagocytosis changes at days 5, 10, 15 and 30
- HLA-DR expression at days 5, 10, 15 and 30
- Incidence of infection
- Incidence of bacteraemia
- Changes in lipopolysaccharide induced monocyte cytokine secretion at days 5 and 15
- Changes in the phenotypic characteristics of lymphocytes at days 5 and 15
- Changes in circulating bacterial 16S-ribosomal DNA (16S-rDNA) at days 5 and 10

3.2.3. Exploratory End Points

Correlation of serum levels of soluble PD-L1 and loss of monocyte phagocytic function in patients with ALF or AD.

4. PARTICIPANT ENTRY

4.1. Pre-Registration Evaluations and Consenting

Potential patients will be identified after admission to hospital for treatment of ALF or AD. They will be identified by clinical hepatology or gastroenterology teams providing care to the patients at the time of admission of clinical diagnosis of ALF or AD. The identification of potential participants will involve reviewing or screening the identifiable personal information of patients. The clinical information of potential patients will firstly be reviewed by the local NHS clinical care team. If acceptable to the patient they will then be approached by the local study team (Local PI, Doctors, Nurses) who will review their medical records to assess suitability for the study. The study team will inform the patients or their personal / nominated consultee about the study. Patients, or their personal / nominated consultee, will receive a written

explanation (Patient Information Sheet and Informed Consent Form/Declaration Form) of the study and must freely give their informed consent in writing prior to any study procedures. If the patient does not have the capacity to consent for themselves, a personal/nominated consultee will be given an information sheet to read and asked to sign a declaration form confirming that in their opinion the patient would be happy to participate in the study. The consent given by the Personal / Nominated Consultee remains valid in law until such time as the patient recovers capacity. At this point, the patient will be informed about the study and asked to decide whether or not they want to continue in the study, and consent to continue will be sought from the patient themselves.

Consent will be taken by the local Study team PI or a clinician designated by the PI.

Following consent, patients will be screened for eligibility for the study and must fulfil the inclusion and exclusion criteria to be admitted into the study, receive study drug and followed-up for further evaluations as per schedule of assessments (see Appendix 1).

Apart from being given one dose of the study drug Nivolumab and having some extra bloods taken, all participants will be treated in the same way as if they were not participating in the study and they will receive standard of care for patients with their condition. There is no expectation that Nivolumab will restore their liver function. Routine clinical procedures such as reporting Hepatitis B virus (HBV) or Hepatitis C virus (HCV) to the health authorities will continue as usual.

Participants will not have their stay in hospital prolonged, nor be subject to additional hospital visits if they are discharged from the hospital before the 30-day period is reached. It is unlikely that the sentinel groups (or participants in the main study) will be in hospital for 12 weeks. They will leave hospital when clinically indicated which may be before day 30. Participant standard of care appointments with their local NHS clinical care team can be integrated with follow-ups by the study team.

4.2. Inclusion Criteria

Inclusion criteria – Group 1 (ALF)

- Male and female patients aged 18 years or older at screening
- Clinical diagnosis of acute liver failure:
 - Presence of jaundice (Bilirubin > 40 uMol/L)
 - INR > 1.5
 - Any degree of encephalopathy
 - No history of cirrhosis or advanced chronic liver disease
- Informed consent (provided by a consultee when patient lacks capacity)

Inclusion criteria – Group 2 (AD)

- Male and female patients aged 18 years or older at screening
- Clinical diagnosis of acute decompensation of cirrhosis including acute-on-chronic liver failure characterised by at least one of:

- Ascites
- Spontaneous bacterial peritonitis
- Encephalopathy (any degree)
- Variceal haemorrhage
- Evidence of cirrhosis based on any of the following:
 - Liver biopsy (at any time)
 - Elastography (at any time) FS > 10KPa
 - Radiological imaging (at any time)
- Informed consent (provided by the patient or a consultee when patient lacks capacity)

4.3. Exclusion Criteria

- Candidates for liver transplantation within 2 months
- Duration of clinically apparent jaundice > 3 months before baseline visit
- Evidence of acute viral hepatitis
- Biliary obstruction
- Hepatocellular carcinoma
- Any known autoimmune disorder, including autoimmune mediated liver failure
- Previous treatment with any checkpoint inhibitor
- Untreated sepsis
- Evidence of current malignancy (except non-melanotic skin cancer)
- Patients with known hypersensitivity or contraindications to anti PD-L1 antibody (Nivolumab)
- Pregnant or lactating women
- Currently enrolled in a CTIMP
- Known HIV infection

Sepsis:

All patients will be screened for infection prior to enrolment. Diagnosis of infection is based on the criteria outlined by Bajaj and colleagues and will involve chest radiography, urinalysis (mid-stream urine (MSU) culture if urinalysis positive)), ascitic tap (if ascites present) and blood cultures if pyrexial. Positive culture and initiation of antibiotics with clinical or radiological signs of infection, as well as clinical suspicion, will be recorded as sepsis.

Blood culture negative pyrexia and a leucocytosis will not be regarded as signs of active sepsis on their own, as these are often co-existent findings with alcohol-related hepatitis. Patients with evidence of sepsis will be treated for a minimum of 2 days with appropriate antibiotics before re-screening. Once the local Principal Investigator (PI) considers that the sepsis is under control, the patient may be re-screened and enrolled if eligible.

Renal Impairment:

Renal impairment and requirement for renal replacement therapy are not contraindications to participation in the study.

4.4. Withdrawal Criteria

Patients who withdraw their consent at any point in the study fall into one of two categories. Site staff should ascertain which category a patient wishes to be in at the point of withdrawal:

a) Those who allow their data (collected up to the point of withdrawal) to be used
OR

b) Those who do not allow the use of any of their data collected prior to withdrawal.

4.5. Patients Lost to Follow-Up

These are patients who do not attend a follow-up assessment after site staff have attempted to contact the patient at least twice, e.g., by telephone.

As these patients have not withdrawn their consent, the data already collected for them may be used and therefore needs returning in the usual manner.

4.6. Patients That Have Died

These patients have not withdrawn their consent and therefore the data already collected for them may be used.

5. ADVERSE EVENTS

5.1. Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded- it should be specified if only some non-serious AEs will be recorded, any reporting should be consistent with the purpose of the study end points.

5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours. However, relapse and death due to acute liver failure or acute decompensation of cirrhosis, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the **<name of REC>** where in the opinion of the Chief Investigator, the event was:

- 'related', i.e., resulted from the administration of any of the research procedures; and
- 'unexpected', i.e., an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs

RGIT@imperial.ac.uk

m.thursz@imperial.ac.uk

(PA: d.campbell@imperial.ac.uk)

**Please send SAE forms to: Professor Mark Thursz, Digestive Diseases
Division, Imperial College,
10th Floor QEQM, South Wharf Rd, Paddington, London W2
Tel: 020 3312 1903 (Mon to Fri 09.00 – 17.00)**

6. ASSESSMENT AND FOLLOW-UP

Please also refer to the tabulated Schedule of Assessments in Appendix 1 which lists all the assessments and indicates with an 'x' at which visits the assessments are performed. Patients due to be discharged will have the samples collected on discharge, as specified in the schedule of amendment below. At a minimum, patients will be contacted for safety evaluations during the 30 days following selection for the study. At this final visit the adverse event and concomitant medications should be reconciled on the eCRF. Documentation of attempts to contact the patient should be recorded in the source documentation.

All patients must have freely given their informed consent before any study-related procedures can be conducted.

Procedures marked '' written in italics are part of routine clinical practice and are listed only to denote that the data will be collected in the eCRF.

6.1. SCREENING

- Informed consent from patient/personal or professional consultee
- Demographic details
- Check Inclusion and Exclusion criteria
- **Hep A, Hep B, Hep C & Hep E screening*
- Pregnancy test (serum or urine)
- **Liver ultrasound (USS)*
- Past medical history including general history using the Charlson Index, liver-specific history (history of jaundice in past 3 months) and smoking history
- Prior/Concomitant medication
- **Complete physical examination, including height, weight, WHO performance status*
- **Vital signs (Temperature, systolic and diastolic blood pressure (BP), pulse rate)*
- **Laboratory tests: haematology, clinical chemistry (including electrolytes, creatinine, eGFR, LFTs [must include AST and GGT]), CRP and prothrombin time*
- **Disease scores: CLIF ...*
- **Encephalopathy Grade*
- **Evidence of GI bleed or sepsis in previous 7 days*
- **Assessment of Acute kidney injury*
- **Infection/ sepsis/ SIRS screening including*
 - *Chest X-ray (CXR)*
 - *Blood cultures*
 - *Ascitic tap (in case required)*
 - *MSU*

6.2. BASELINE

- Inclusion and Exclusion criteria
- Concomitant medication
- **Vital signs (Temperature, systolic and diastolic blood pressure (BP), pulse rate)*
- **Laboratory tests: haematology, clinical chemistry (including electrolytes, creatinine, eGFR, LFTs), CRP and prothrombin time*
- **Disease scores: CLIF / MELD*
- **Evidence of GI bleed or sepsis in previous 7 days*
- **Acute kidney injury*
- **Encephalopathy Grade*
- EDTA sample for host & bacterial DNA
- Serum sample for sPD-L1
- Heparinised sample for PBMC (Monocyte phagocytosis, Monocyte phenotype, Lymphocyte phenotype)
- Heparinised sample for PBMC (Gene expression)

- Duration since admission to hospital
- Adverse events
- Stool sample
- Administration of study drug

If baseline visit is performed more than 48 hours after screening, then sepsis screening should be repeated.

6.3. EVALUATIONS DURING AND AFTER TREATMENT

Treatment Day 5, 10, 15 Assessments.

- Concomitant medication
- **Vital signs (Temperature, systolic and diastolic blood pressure (BP), pulse rate)*
- **Laboratory tests: haematology, clinical chemistry (including electrolytes, creatinine, eGFR, LFTs), CRP and prothrombin time*
- **Disease scores: CLIF / MELD*
- **Evidence of GI bleed or sepsis in previous 5 days*
- **Acute kidney injury*
- **Encephalopathy Grade*
- EDTA sample for host & bacterial DNA
- Serum sample for SPD-L1
- Heparinised sample for PBMC (Monocyte phagocytosis, Monocyte phenotype, Lymphocyte phenotype)
- Adverse events

6.4. DAY 30

- Concomitant medication
- **Vital signs (Temperature, systolic and diastolic blood pressure (BP), pulse rate)*
- **Laboratory tests: haematology, clinical chemistry (including electrolytes, creatinine, eGFR, LFTs), CRP and prothrombin time*
- **Disease scores: CLIF / MELD*
- **Evidence of GI bleed or sepsis in previous 7 days*
- **Acute kidney injury*
- **Encephalopathy Grade*
- EDTA sample for host & bacterial DNA
- Serum sample for SPD-L1
- Heparinised sample for PBMC (Monocyte phagocytosis, Monocyte phenotype, Lymphocyte phenotype)
- Duration since admission to hospital
- Adverse events

6.5. WEEK 12 (SENTINEL COHORTS ONLY)

- Concomitant medication

- **Vital signs (Temperature, systolic and diastolic blood pressure (BP), pulse rate)*
- **Laboratory tests: haematology, clinical chemistry (including electrolytes, creatinine, eGFR, LFTs), CRP and prothrombin time*
- **Disease scores: CLIF / MELD*
- **Evidence of GI bleed or sepsis in previous 7 days*
- **Acute kidney injury*
- **Encephalopathy Grade*
- Heparinised sample for PBMC (Monocyte phagocytosis, Monocyte phenotype, Lymphocyte phenotype)
- Duration since admission to hospital
- Adverse events

6.6. Patient demographics/other baseline characteristics

Patient demographic and baseline characteristic data to be collected on all patients includes: age, gender, ethnicity, source of patient referral, relevant medical history/current medical condition present before signing informed consent. Where possible diagnoses but not symptoms will be recorded. **All demographic data will be recorded by the local study team (PI, study doctors, study nurses).**

Detailed information on the patient's vaccination status (to be recorded as part of prior medication), disease diagnosis and disease related prior medications, surgical and medical procedures that occurred before signing informed consent will also be collected.

Investigators will have the discretion to record abnormal test findings on the medical history CRF whenever in their judgment, the test abnormality occurred prior to the informed consent signature.

6.7. Physical exam

A complete physical examination will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, vascular and neurological. If indicated based on medical history and/or symptoms, rectal, external genitalia, breast, and pelvic exams will be performed. Weight and height will be measured as per the visit schedule. Physical exam includes WHO performance status (Appendix 2). **This complete physical examination will be undertaken by the local study team (PI, study doctors, study nurses).**

6.8. Vital signs

Vital signs will include blood pressure (BP), pulse and body temperature measurements. Systolic and diastolic BP and radial pulse rate will be assessed after the patient has rested in the supine position for at least 3 minutes. Blood pressure

should be assessed on the same arm each time measurements are taken. Body temperature should be measured by validated thermometers as commonly used by sites in the respective patient population. The same type of thermometer should be used throughout the study. The vital signs assessment will be undertaken by the local study team (PI, study doctors, study nurses).

6.9. Sample Collection and Storage

6.9.1. Research Sample Labelling

All research samples should be labelled with the following information:

- NORMALISE Study
- Sample type: Serum/EDTA/PBMC/Urine
- Participant Study ID
- Collection Date

6.9.2. Research Sample Storage

All patient samples collected at the 2 recruiting NHS sites will be stored at the local NHS site for the short-term before being shipped via courier to the Hepatology Department on 10th Floor QEQM Wing at St Mary's Hospital, Paddington. This is the laboratory of the CI, Prof Mark Thursz and samples will be stored at this location for the duration of the study. Sample analysis by the Imperial College London study team will also take place at this location. In the long term the samples will be stored within the Hepatology Departmental Tissue Bank at Imperial College at St Mary's (10th Floor QEQM). This Tissue Bank fully conforms to HTA regulation. All freezers are constantly monitored by T-Scan alarm systems and are fully secured. Access to samples are by permission of the study CI and freezers are locked and are kept in rooms which are locked and entry is by authorised pin code.

At the end of the research samples will be transferred into the Imperial College Hepatology and Gastroenterology Biobank which operates under the licence of Imperial College. Patients will be asked to consent to the storage of samples for future unspecified use. They will be stored either under the Tissue Bank licence or under ethical approval for future use in further research projects.

6.9.3. Blood Tests

All patients will have blood tests taken for routine care, for serum samples, for DNA sampling and for PBMC samples. Routine blood tests will be drawn by the NHS hospital ward staff or a research nurse. Blood tests for serum, DNA and PBMCs will be drawn at the same time as routine tests if possible.

6.9.4. Standard laboratory tests

The haematology panel will include: Haemoglobin, white blood cell count with differential including lymphocytes, monocytes, neutrophils, platelet count and will be

measured. Absolute Neutrophil Count (ANC) will be determined by the laboratory. Prothrombin time will be assessed as well.

The clinical chemistry panel will include: alkaline phosphatase, total bilirubin, total cholesterol, LDL-C, HDL-C, creatinine, creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), α -amylase, sodium, potassium, lactate dehydrogenase (LDH), triglycerides and CRP; The estimated creatinine clearance will be calculated using the Schwartz formula for each creatinine measurement. This will require the information on patient's age, sex, weight, height and serum creatinine levels.

Urine samples will be assessed: Urinalysis (MSU) + assessment for urinary tract infection (UTI); urine white blood cell count with either positive urine gram stain or culture.

6.9.5. Disease Severity Scores

Standard laboratory assessments will be used to determine:

Model for End-Stage Liver Disease (MELD) (all patients)

MELD Score = $10 * ((0.957 * \ln(\text{Creatinine})) + (0.378 * \ln(\text{Bilirubin})) + (1.12 * \ln(\text{INR}))) + 6.43$ calculated using mdcalc.com

CLIF score (Acute Decompensation)

Based on Age, White blood cell count, bilirubin, creatinine, INR, mean arterial pressure (MAP), grade of encephalopathy, PaO₂, FiO₂ and calculated using mdcalc.com

King's College Criteria

Based on Arterial pH, INR, Creatinine, grade of encephalopathy, lactate calculated using mdcalc.com

SIRS

Recommendations of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference; Presence of 2 or more criteria out of following:

Temperature < 36 °C or > 38 °C

Heart rate > 90 beats/minute

Respiratory rate > 20 breaths/minute or venous pCO₂ <32 mmHg

Leukocyte count > 12,000/mm³ or < 4,000/mm³ or band forms > 10%

6.9.6. Infection screening

Infections:

Bacteraemia will be determined using standard blood culture tests with data extracted from the hospital laboratory record.

Incidence of infection will be determined in two ways: 1. definite infection using criteria specified by the North American Consortium for the Study of End Stage Liver Disease (12) and 2. suspected infections where antibiotics are initiated therapeutically in the absence of positive bacterial cultures.

Diagnosis of infection is based on the criteria outlined by Bajaj and colleagues: 1) spontaneous bacteraemia: positive blood cultures without a source of infection; 2) SBP: ascitic fluid polymorphonuclear cells $>250/\mu\text{L}$; 3) lower respiratory tract infections: new pulmonary infiltrate in the presence of: i) at least one respiratory symptom (cough, sputum production, dyspnoea, pleuritic pain) with ii) at least one finding on auscultation (rales or crepitation) or one sign of infection (core body temperature $>38^{\circ}\text{C}$ or less than 36°C , shivering, or leukocyte count $>10,000/\text{mm}^3$ or $<4,000/\text{mm}^3$) in the absence of antibiotics; 4) *Clostridium difficile* Infection: diarrhoea with a positive *C. difficile* assay; 5) bacterial entero-colitis: diarrhoea or dysentery with a positive stool culture for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, or pathogenic *E. coli*; 6) soft-tissue/skin Infection: fever with cellulitis; 7) urinary tract infection (UTI): urine white blood cell $>15/\text{high-power field}$ with either positive urine gram stain or culture; 8) intra-abdominal infections: diverticulitis, appendicitis, cholangitis, etc.; 9) other infections not covered above; and 10) fungal infections as a separate category. Infection screening will involve chest radiography, urinalysis (mid-stream urine (MSU) culture if urinalysis positive), ascitic tap (if ascites present) and blood cultures if pyrexial.

6.9.7. Monocyte HLA-DR and Phagocytosis

HLA-DR expression will be measured using flow cytometry after staining with mouse anti-human HLA-DR (Invitrogen 45-9956-42).

In order to measure phagocytosis we will use flow cytometry to measure bacterial internalization by peripheral blood monocytes using pHrodo *E. coli*, a pH-sensitive assay indicating phagolysosome acidification. This assay will exactly complement the ex-vivo work we recently published.

Measurement of the change in phagocytosis in response to systemic anti-PD1 antibody treatment will be made at days 2, 5, 10 and 15 to explore the timecourse of the immunological effect.

6.9.8. Bacterial 16S-rDNA

Bacterial 16S-rDNA will be measured using the real-time PCR assay based on primers located in v7-v9 variable region (3). 16S-rDNA will be measured at baseline, days 5, 10 and 15. This analysis will be performed by the study team at Imperial College London.

6.9.9. Immune cell characterization:

To determine whether anti-PD1 treatment restores monocyte function we will conduct LPS stimulation in vitro assays before and after systemic treatment to measure inflammatory cytokine levels. In parallel, we will phenotypically characterise peripheral monocytes and lymphocytes, before and after systemic anti-PD1

treatment, using flow cytometry to examine various activation and co-stimulatory markers (e.g., CD80, CD-86, HLA-DR and CD25, CD69).

Further analysis of monocyte phenotype will be undertaken by mRNA expression analysis before and after treatment with anti-PD1 antibody. A separate aliquot of peripheral blood mononuclear cells will be used to isolate CD14 positive monocytes and gene expression analysed using the NanaoString nCounter system. **This analysis will be performed by the study team at Imperial College London.**

6.9.10. Soluble PD-L1:

In order to explore the utility of sPD-L1 as a marker of infection risk and potential response to anti-PD1 treatment, the levels of this biomarker will be measured at the time of admission in a large cohort of patients with ALF, acute decompensation and alcohol-related hepatitis. Baseline levels of sPD-L1 will be correlated with clinical outcomes and the incidence of infection and mortality. **This analysis will be performed by the study team at Imperial College London.**

6.9.11. Other assessments

- HIV test: A HIV test will be performed at screening (HIV test conducted within 6 months of screening may be accepted). Patients with a known infection with HIV at screening or randomisation will be excluded from participating in the study. **This assessment will be undertaken by the local study team (PI, study doctors, study nurses).**
- Pregnancy Test: A pregnancy test will be performed either as urine or serum β -hCG test at screening. Patients who are pregnant or lactating are not eligible to participate in the study. **This assessment will be undertaken by the local study team (PI, study doctors, study nurses).**
- Hepatitis A, B, C & E Serology: Serology will be undertaken at screening and evidence of active viral hepatitis will exclude the patient from participation. **These assessments will be undertaken by the local study team (PI, study doctors, study nurses).**

6.10. Follow-Up

Patients recruited to the sentinel cohorts will be followed-up for 12 weeks following treatment with Nivolumab. Patients recruited to the main study will be followed up for 30-days following treatment with Nivolumab. All patients will receive a single dose of Nivolumab. Samples will, wherever possible, be collected from participants when they would be having blood samples taken as part of their standard clinical care either as an inpatient or at an outpatient visit if they have been discharged.

Participants in the study will not have their stay in hospital prolonged, nor be subject to additional hospital visits if they are discharged from the hospital before the 30-day period is reached. Their standard of care appointments with their local NHS clinical care team will be integrated with follow-ups by the study team.

If the patient consents, it is the investigator's responsibility to inform the subject's General Practitioner by letter that the subject is taking part in the study provided the subject agrees to this, and information to this effect is included in the Patient Information Sheet and Informed Consent. A copy of the letter should be filed in research record for the patient at sites.

The local study PI and his / her study team will remain in regular contact with the participant over the course of their participation in the study. Should any new information come available the clinical team will communicate this to the participant.

6.11. Incidental Findings

Should any incidental findings be identified by the local study team PI and his / her clinical team, the CI will review these and report the findings to the participants GP and Data Monitoring and Ethics Committee.

6.12. End of study

The recruitment period of the study will be stopped when:

- The stated number of patients to be recruited is reached, or the study is stopped for another reason, e.g., by the funding body or the regulatory authority.

The active treatment phase will be completed:

- 28 days after start of treatment of the last patient randomised.

The end of the study is defined as:

- The end of the study will occur when the final participant has completed the last study visit and all study data have been captured on the study database.

Regardless of the reason for termination, all data available for the patient at the time of discontinuation of follow-up must be recorded in the CRF. All reasons for discontinuation of treatment must be documented.

In terminating the study, the Sponsor and the Investigators will ensure that adequate consideration is given to the protection of the patient's interests.

7. STATISTICS AND DATA ANALYSIS

A sample size and power calculation was conducted to estimate the number of patients required for the primary endpoint, change in HLA-DR expression, in the acute liver failure arm. In patients with acute liver failure the mean HLA-DR expression is 1477, with a standard deviation of 500. In a sepsis study the response to PD-L1 inhibition increased HLA-DR by 100% (range 32 – 153%) with a standard deviation of the difference in means being 500 (10). In this study HLA-DR expression did not change at 15 days in the control population. Based on this data and assuming a conservative 30% increase in HLA-DR expression would be sufficient to improve immune function.

Using STATA 17 with the following assumptions:

- effect size of 30% increase in pre-treatment HLA-DR,
- power = 90%,
- significance = 0.05,
- mean pre-treatment HLA-DR= 1477 (SD= 551),
- difference between pre-treatment and post-treatment HLA-DR = 443,
- standard deviation of the difference in means = 500,
- one-sided paired t-test

The team estimate that the sample size required is 13 patients. Taking into consideration 30% loss of follow up at 15 days the team will recruit 19 patients with acute liver failure across the two sites.

The primary analysis of the primary endpoint, change in HLA-DR expression, will be a one-sided paired t-test to compare baseline with the measurement of HLA-DR expression in peripheral blood monocytes at day 15 after treatment with study drug.

The secondary analysis of the primary endpoint will investigate changes over time from baseline using a linear mixed model.

The main secondary endpoint will be the incidence of infection after treatment. In patients with acute decompensation the expected rate of infection is 35%. In order to show an absolute 50% reduction in infection with 90% power ($\alpha = 0.05$), using a one-sided one-sample proportion test, we need to recruit 53 patients. We aim to recruit 59 acute decompensation patients to allow for a possible 10% loss to follow up.

A statistical analysis plan will be drafted prior to data lock and commencement of the analyses. This will outline the detailed plan for analysis for primary and secondary outcomes.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY, ETHICAL AND LEGAL ISSUES

8.1. Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the 7th revision of the 1964 Declaration of Helsinki.

8.2. Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

8.3. Non-Compliance and Serious Breaches

All protocol deviations and protocol violations will be reported via the eCRF/CRF and reviewed by the Chief Investigator and Study Manager on a monthly basis. Protocol violations will be reported to the Sponsor.

An assessment of whether the protocol deviation/violation constitutes a serious breach will be made.

A serious breach is defined as:

A breach of the conditions and principles of GCP in connection with a study or the protocol, which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the UK study subjects; or
- The overall scientific value of the study

The Sponsor will be notified within 24 hours of identifying a likely Serious Breach. If a decision is made that the incident constitutes a Serious Breach, this will be reported to the REC within 7 days of becoming aware of the serious breach.

8.4. Ethics Approval

The Study Coordination Centre has obtained approval from the **xxx** Research Ethics Committee (REC) and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out.

The HRA and all participating sites will be notified of all protocol amendments to assess whether the amendment affects the institutional approval for each site.

8.5. Informed Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and patients will be given 24 hours to consider the study. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study, the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he / she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases, the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

Informed consent will be obtained before researchers outside of the normal care team access identifiable personal information. Medical records and other personal data generated during the study may be examined by representatives of the Sponsor (Imperial College) and representatives of Regulatory authorities.

Arrangements will be available for persons who might not adequately understand verbal explanations or written information given in English, or who have special communication needs. If required, information on the study will be given, and consent will be obtained, with the use of interpreters or translators according to the local hospital policy.

- **Incapacitated Patients**

Potential patients for the study who present with hepatic encephalopathy may be unable to consent for themselves but are not excluded from the study. Special arrangements are in place to ensure that the interests of such patients are protected.

When considering a patient who is unable to consent for themselves for suitability for the study, the decision on whether a patient would be happy to partake, or refuse to partake, in a study will be taken by a “nominated consultee” who is independent of the research team and should act on the basis of the person’s presumed wishes. The appropriate consultee will be provided with the approved Nominated Consultee Information Sheet and Consultee Declaration Form, to document the process.

The consent given by the nominated consultee remains valid in law until such time as the as the patient recovers capacity. At this point, the patient will be informed about the study and asked to decide whether or not they want to continue in the study, and consent to continue will be sought from the patient themselves.

8.6. Contact with General Practitioner

It is the local study site PI’s responsibility to inform the participant’s General Practitioner (where applicable) by letter that the participant is taking part in the study provided the participant agrees to this, and information to this effect is included in the Participant Information Sheet and Informed Consent. A copy of the letter should be filed in the Investigator Site File.

8.7. Data Protection and Participant Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. The local study team PI must ensure that the participant’s confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, participants will be identified by a participant ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the local study team PI.

The local study team PI shall permit direct access to participants’ records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor and REC.

Only members of the patient's existing clinical care team at their NHS site will have access to patient records and personal data without explicit consent. They will identify potential participants and make the initial approach to patients. All patient data will be treated confidentiality. Patients will be fully informed on the patient information sheets and consent forms of how their data will be used and who will have access to it. This will be in line with General Data Protection Regulation (GDPR).

8.8. Study Documentation and Data Storage

Identifiable patient data will only be stored on secure NHS computers which may only be accessed by the clinicians involved in the patients' clinical care. The study database will assign a unique identifying numerical code which is distinct from the NHS number of the hospital record number, referred to as the Patient Study ID number. The unique identifier will be used for all research data stored on investigators computers. This pseudonymised data will be kept on NHS and University computers. Such data will be encrypted to the local ICT requirements. Paper records (consent forms etc) will be stored securely on NHS premises. Information gleaned from access will remain entirely confidential and will only be recorded anonymously in study records.

The CI must retain essential documents including personal data that will be stored and accessed after the study has ended until notified by the Sponsor, and for at least 10 years after study completion. Once the study has ended all study documentation will be sent to Imperial College Archive Unit and held under the name and responsibility of the Chief Investigator.

Subject files and other source data (including copies of protocols, CRFs, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be retained. Documents should be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

8.9. Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.10. Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.11. Funding

The Medical Research Council (MRC) are funding this study.

8.12. Audits

The study may be subject to audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through the Chief Investigator and Dr Emma Lord (e.lord@imperial.ac.uk).

Chief Investigator

Professor Mark Thursz
Digestive Diseases Division, Imperial College,
10 S Wharf Rd, Paddington, London W2

Contact person:

Ms. Dawn Campbell
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10. DATA MANAGEMENT

CRFs will be in English. Generic names for concomitant medications should be recorded in the CRF wherever possible. All written material to be used by subjects must use vocabulary that is clearly understood and be in the language appropriate for the study site.

Data will be collected through the electronic case report forms (eCRF) on the NORMALISE database at each patient visit as described in the scheduled of assessments (Appendix 1). Details of procedures for eCRF completion will be provided in a study manual. At the end of the study the database will be locked, and the custodian of the data generated by the study will be the CI at Imperial College London. Data generated by the study will be analysed by statisticians within the King's College London statistics team and the CI's study team at Imperial College London. All analysis (statistical and otherwise) will take place on secure university computers. The research teamwork with the Information Governance team at Imperial College to ensure that appropriate measures and systems are in place to safeguard the security of the data, during analysis and storage. All data held on the university computers will be pseudonymised with the unique participant identification number. Only fully anonymised data will be published.

Explicit consent for access to medical records by members of the research team at Imperial College London and King's College London will be gained. Transfer on magnetic/optical media or networks will only be in encrypted form, according to local NHS ICT protocols. Where data is stored on NHS computers, appropriate access controls will be in place to ensure that access to confidential research information is

restricted to those who need access. Medical records and other personal data generated during the study may be examined by representatives of the sponsor (Imperial College), by people working on behalf of the sponsor, and by representatives of Regulatory authorities, where it is relevant to this research. Where access to participants' personal data is required during the study by members of the research team at Imperial College London or King's College London, explicit consent for this access will be sought from each participant or personal / nominated consultee on the consent forms.

11. PUBLICATION POLICY

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

Therefore, all information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) are completed.

The results may be published or presented by the investigator(s), but the Sponsor will be given the opportunity to review and comment on any such results before any presentations or publications are produced.

Authorship will be determined according to the internationally agreed criteria for authorship (www.icmje.org). Authorship of parallel studies initiated outside of the The MRC, as study funder, will be informed of study publications as per the contract between the MRC and Imperial College.

Results of the study will be disseminated via peer-reviewed scientific journals, internal reports, conference presentations and publications on websites for Imperial College London and King's College London.

Patients will be aware of their own clinical condition following treatment and participation in this study, but they will not automatically be told of the study-wide results. However, if patients specifically ask to be advised of the research results, then they will be contacted at the end of the study by the study team and will be informed.

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Appendix 1. Summary of investigations, treatment, and assessments

	Screening	Baseline /Day 1	Da y 5	Da y 10	Da y 15	On Discharge	Day 30	¹ Week 24
Informed Consent	X							
Demographic data	X							
Inclusion/Exclusion criteria		X						
Pregnancy test	X							
Viral Hepatitis serology	X							
Duration since admission to hospital		X				X		
Medical history	X							
Prior/Concomitant medications	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Physical exam		X					X	
Weight	X					X	X	
Height	X							
Encephalopathy grade	X	X	X	X	X	X	X	X
Administration of study drug		X						
Prothrombin time/INR	X	X	X	X	X	X	X	X
Haematology	X	X	X	X	X	X	X	X
Clinical chemistry	X	X	X	X	X	X	X	X
Severity scores: CLIF/SOFA / MELD/KCH	X	X	X	X	X	X	X	X
Overt GI Haemorrhage	X	X	X	X	X	X	X	X
Acute kidney injury	X	X	X	X	X	X	X	X
Infection/ Sepsis/ SIRS	X		X	X	X	X	X	
16S-rDNA (EDTA sample)		X	X	X	X	X	X	
Monocyte function (PBMC sample)		X	X	X	X	X	X	
Soluble PD-L1 (serum sample)		X			X			
Gene expression sample		X			X			
Adverse events		X	X	X	X	X	X	X

- 1 Required for patients in the sentinel cohorts.
- 2 CLIF-C ACLF score for acute decompensation or SOFA and Kings College Criteria for acute liver failure. All scores to be calculated using the mdcalc.com website.

Appendix 2. WHO Performance status

0	Asymptomatic (Fully active, able to carry on all pre-disease activities without restriction)
1	Symptomatic but completely ambulatory (Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work)
2	Symptomatic, <50% in bed during the day (Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours)
3	Symptomatic, >50% in a chair or in bed, but not bedbound (Capable of only limited self-care, confined to bed or chair 50% or more of waking hours)
4	Bedbound (Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair)
5	Death